Multimodality Molecular Imaging of Stem Cells Therapy of Myocardial Infarction

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Abstract: Stem cell therapy in patients with acute myocardial infarction promises a new exciting option. However, the long term fate of stem cells transplanted into myocardial is not clear. Molecular imaging play a vital role in tracking stem cells after intramyocardial delivery as well as in helping us understand the mechanisms of stem cell biology in vivo. In this review, we summarize the principles underlying four kinds of stem cells applied into myocardial infarction and the major tracking methods. Finally, we discuss that molecular imaging will be useful to the successful clinical implementation of this novel therapy.

***Keywords*:** myocardial infarction, stem cells, imaging, positron emission tomography, magnetic resonance imaging, bioluminescence

Introduction

Coronary artery disease (CAD) is one of the most important reasons of threating to human health which is a leading cause of morbidity and mortality worldwide. Ischemic insult to the myocardium during the course of CAD causes cardiomyocyte death, loss of functioning cardiomyocytes, decrease blood supply to the heart, leading to deposit fibrous tissue and adverse ventricular remodeling [1]. Currently, therapeutic measures to improve circulating function such as interventional treatments, drug therapy, and surgery, which are limited because of the inability to save damaged or death myocardial cells and the faintness of strength of reversing deterioration of the cardiac function. Heart transplantation is the new method of saving the patients, life of the CAD. However, heart transplantation cannot be widely used in clinical on account of the many defects, such as lacking of donor, the high cost of surgery, the serious damage of patients, bodies and the inevitable immunological rejection. The treatment for CAD is urgently needed, that is, replace necrotic myocardial cells with new healthful cardiomyocytes. In recent years, transplantation of stem cells promises to a new option in the therapy of CAD owing to stem cell has novel regenerative capacity. Many studies have shown that stem cells injected into injured territory can enhance myocardial function [2]. Although clinical and preclinical trials both prove feasibility and safely, its clinical application has been limited extremely because of the behavior and long-term fate in vivo after intramyocardial delivery remain a mystery. It is essential for determining the mechanisms of cell therapy to understand the dynamics of cell trafficking and the long-term rates of cell survival and engraftment. Thus, non-invasive imaging in vivo is important of monitoring or tracking the fundamental behavior of stem cells. Here, we discuss state-of-art stem cells and trafficking imaging methods.

1. Stem cells of the treatment of myocardial infarction

1.1 Embryonic Stem Cells (ESCs)

ESC is the hot topic in the fields of medicine and biology, which has been regarded as the holy grail of myocardial infarction therapy with promises to novel regenerate cardiac tissue. In 1981, Evans and Kaufman first harvested from the inner cell mass (ICM) of the mouse blastocyst [3, 4]. They have the most pluripotent potential of any cell types under defined conditions and possess totipotency. The first human ESC (hESC) lines were successfully generated by Thomson JA and his colleagues from in vitro fertilized embryos in 1998 [5]. hESC has given medical field the tantalizing prospect of differentiation into any tissue cells controlled. ESCs possess unique properties of unlimited proliferation in vitro, self-

renewal capacity and pluripotency that means they can able to differentiate into any cell types of adult tissues, but cannot solely develop into individuals. Consequently, ESCs have been touted as a dominant candidate source for novel regenerative medicine therapies, and under defined conditions have been used to derive various cell populations such as myocardial, endothelial and neurocyte cells. Cells derived from ESCs will be allogeneic and their use is likely to require the co-administration of immunosuppressive agents, which carry their own substantial risks [6].

1.2 Mesenchymal stem cells (MSCs)

MSCs or multipotent mesenchymal stromal cells are a heterogeneous group of non-hematopoietic cells that can be obtained from bone marrow aspirates or adipose tissues, which were isolated from mesoderm or ectoderm. MSCs were described firstly by Friedenstein et al. in 1974 that could regenerate in vivo [7]. MSCs hold the great attention and interest for their potential therapeutic effects. There are increasing proofs that adult stromal stem cells and progenitor cells that can be used for therapy of various diseases. MSCs or multipotent marrow stromal cells that are found in adult donor bone marrow are self-renewing, pluripotent adult stem cells and that can differentiate into cell types of mesenchymal lineage, including bone, cartilage, and adipose tissues [8, 9]. MSCs also have highly immunosuppressive capabilities.

1.3 Adipose derived stem cells (ADSCs)

Adipose tissue includes a large portion of stem cells and contains many cells types, for example, preadipocytes, adipocytes, vascular endothelial cells and vascular smooth muscle cells. These cells possess unique characteristics of proliferation ability and multipotent capacities [10]. They can gradually differentiation into multiple mesenchymal cells such as chondrogenic, osteogenic, adipogenic and myogenic potentials under certain controlled conditions in vivo or in vitro. ADSCs are also able to express numerous growth factors, including vascular endothelial growth factor and hepatocyte growth factor [11]. However, ADSCs in the emerging field of regenerative medicine is being critically evaluated as a source for stem cells and a useful tool in biotechnology, it must be pointed out that the nature of ADSCs and their differentiation stage remain unknown. Wherefore, the biological safety of ADSCs implantation demands a lot of preclinical trials.

1.4 Induced pluripotent stem cells (iPSCs)

It is the revolutionary breakthrough that Professors Gurdon and Yamanaka illuminated in 2006 that both mouse fibroblasts by retroviral transduction of just four genes (Oct3/4, Sox2, c-Myc and Klf4), could be reprogrammed to a pluripotent state similar to that observed in ESCs [12]. The reprogrammed cells, known as iPS cells, own many unique features of ES cells, and represent one of the novel promising sources of regenerative medicine. These landmark advances in basic cell biology were celebrated by the recent award of the Nobel Prize for Physiology & Medicine to Professors Gurdon and Yamanaka [13]. By transduction of specific factors, iPSCs are isolated from adult somatic cells [14]. Given their unlimited proliferation and differentiation potential, iPSCs represent promising sources for cell therapy and tools for research and drug discovery. Since the major discovery of iPSCs, several of studies in this field at surprising rate have developed. The discovery of iPSCs detours ethical, legal and political issues. However, the biggest challenge remains the potential risk of inducing cancer. Biologic safety especially formation of teratoma result from cell population transplanting is the most important problem. These kinds of stem cells have owning advantages and disadvantages, therefore, stem cells would be choose according to clinical and preclinical applications.

2. Multimodality molecular imaging for tracking stem cells in cardiac repair

The alchemy of stem cell therapies holds the novel promise and attention among clinicians, patients and scientists for cardiac regeneration. Result from the absence of explicit or standardized protocols, consequences have been mixed[15]. Furthermore, clinical application of stem cell therapies has been limited extremely because of the behavior and long-term fate in vivo after intramyocardial delivery remains a mystery. To determine the mechanisms of cell therapy, it is important to understand the dynamics of cell trafficking and the long-term rates of cell survival and engraftment. We need a tool not only for monitoring the long-term fate of stem cells transplanted into ischemic setting but also assessing the recovery of cardiac function. Thus, non-invasive in vivo imaging is important of monitoring or tracking the fundamental behavior of stem cells. Molecular imaging plays a critical role for understanding the dynamics of cell trafficking and the long-term fate of cell survival and engraftment of stem cell therapy. Here, we discuss state-of-art stem cells and trafficking imaging methods.

2.1 Positron emission tomography (PET)

PET is one of the most sensitive imaging modalities of non-invasive tracking of stem cells, which have two main classic strategies: direct stem cell labeling and reporter-gene imaging [16].

Direct stem cell labeling is a traditional method of monitoring cells in vivo. Cells are incubated with a radiotracer that allows lipophilic molecules to diffuse and be "trapped" into the cells. After a short incubation period, the cells are washed

to remove any unbound activity and are then injected into the host[17]. The results of labeling technology depend on cell types and preparation techniques that is incubation time, environment, and radiotracer characteristics and concentrations [18]. The advantages of direct labeling of stem cells are used to offer information on the homing and the biodistribution of cells injected into host. It must be point out that the major obstacles include dilution of signal from cell division and the absence of ability to determine cell function and viability.

Reporter gene imaging has been used to investigate stem cell fate, destiny, engraftment and migration in vivo. Reporter genes can be introduced into stem cells prior to implantation by transfection of cDNA (complementary DNA) plasmids or by transduction into the host cell genome via viral vectors [19]. Constitutive expression of reporter genes is driven by endogenous or exogenous gene promoters (e.g. viral promoters of lentiviruses) and requires stable translocation into cells. This approach consists of two main modes, namely enzyme-based and transporter- and receptor-based approaches.

2.2 Magnetic resonance imaging (MRI)

MRI, which is a frequently used tool for in vivo imaging of transplanted cells, was among the first imaging modalities applied for monitoring the cellular actions of stem cells in vitro and in vivo. Its main advantages offer anatomic resolution and assessing recovery of cardiac function. It achieves the dream of supplement detailed morphological and functional information of heart following inject stem cells into injured areas. In fact, gadolinium and super paramagnetic iron oxide (SPIO) are agents that can be used for direct stem cell labeling [1]. Moreover, MRI has been reported for monitoring the long term-fate of stem cells in vivo [20]. Even though MRI can depict three-dimensional spatial information, the lack of high sensitivity and quantifying a labeled cell population are limited this technology widely used. In addition, SPIO may lead stem cells to death. It may not be possible to determine whether the MR signal originates from viable cells or from the persistence of the cell label after cell death.

2.3 Optical imaging

Bioluminescence imaging (BLI) and fluorescence imaging (FLI) are commonly used for tracking stem cells in preclinical models. BLI is usually used for studying the behavior of stem cells transplanted [21]. Stem cells which transduced with a luciferase gene implanted in the recipient animal. BLI have high sensitive, simple, inexpensive and quantitative. However, to date, it limit into preclinical test result from high rates of scattering of visible wavelength photons on the human scale, poor tissue penetration (1-2 cm) (allowing only surface imaging) and low resolution (3-5 mm) [22]

Each molecular imaging modality has its advantages and disadvantages, and combining multiple imaging modalities may provide complementary information than a single modality alone. Therefore, multimodal molecular imaging is evolving as an indispensable tool for tracking stem cells.

3. Discussion

Currently, therapeutic measures to improve circulating function such as interventional treatments, drug therapy, and surgery, which are limited because of the inability to save damaged or death myocardial cells and the faintness of strength of reversing deterioration of the cardiac function. However, heart transplantation cannot be widely used in clinical on account of the many defects, such as lacking of donor, the high cost of surgery, the serious damage of patients，bodies and the inevitable immunological rejection of transplantation. The treatment for CAD is urgently needed, that is, replace necrotic myocardial cells with new healthful cardiomyocytes. In recent years, stem cell transplantation promises to a new option in the therapy of CAD owing to stem cells has novel regenerative capacity. Many studies have shown that stem cells injected into injured territory can enhance myocardial function [2].

3.1 ESC and myocardial infarction

The discovery of ESC has given the tool available to medical scientists for myocardial infarction. Ischemic insult to the myocardium causes cardiomyocyte death, loss of functioning cardiomyocytes, decrease blood supply to the heart, leading to deposit fibrous tissue and adverse ventricular remodeling [1]. Therefore, ESC could be regarded as a novel source of regeneration cardiomyocytes. Many groups have shown that murine ESCs can improve cardiac function in mice and rats following an myocardial infarction, fortunately had no evidence of teratoma formation, arrhythmias, immunology rejection, ventricular ectopy [23-26]. Nevertheless, these researches relied on postmortem analysis at different time points, such as green or cyan fluorescence markers, which demanded the experimental animals to be killed. So, the behavior and long-term fate in vivo after intramyocardial delivery remain a mystery. Molecular imaging would provide a better understanding and monitoring of cells in the evaluation of the functional effects of ESC grafting. Two approaches are imaging of stem cells radiolabel with 18F-FDG detected by PET and iron-labeled using MRI. However, the limitation of the short half-life of 18F (~110 min) hamper collection of the biological and mechanistic data [27].

3.2 MSC and myocardial infarction

MSCs have a limited differentiation potential toward cardiomyocytes. Intramyocardial mononuclear bone marrow cell (BMC) transplantation promise a new treatment modality in patients after myocardial infarction [28] or with therapy-refractory myocardial ischemia [29]. In addition to these studies, several preclinical studies and clinical trials have shown that MSC attenuate maladaptive left ventricular (LV) remodeling, and preserve and/or promote recovery of pump performance after myocardial infarction (MI) [30, 31]. The mechanism of improving cardiac function has been variously attributed to de novo cardiomyogenesis, and/or neoangiogenesis. An increasing number of evidence suggests, however, that the therapeutic effects of MSC transplantation primarily result from indirect stimulation (often termed paracrine) of neovascularisation and protection from ischemia-induced apoptosis [31, 32]. The mechanism of this effect was hMSCs differentiated into endothelial cells and integrated into blood vessels after MI, increased angiogenesis and decreased fibrosis were associated with cardiac functional improvement following hMSC transplantation. Understanding the dynamics of cell trafficking and the long-term fate of cell survival and engraftment is essential to determine the mechanisms of cell therapy. The single imaging modality used to monitor cardiomyocyte therapy has its drawbacks in tracking the long-term fate of trafficked cells. Thus, development of multimodal imaging of reporter genes is necessary to play an important role in tracking MSCs in vivo.

3.3 ADSCs and myocardial infarction

Adipose tissue-derived stem cell, owning its easy obtainment, self-renewal capacity, and low immunosuppression, has been widely used in both basic research and clinical translation [33]. Jun-jie Yang et al. [34] have found that ADSCs holding great potential for the transplantation in treating heart disease. In this study, 5 × 106 ADSCs with a lentiviral vector carrying a triple-fusion reporter gene that consists of firefly luciferase, monomeric red fluorescence protein, and truncated thymidine kinase (fluc-mrfp-ttk) were injected along the border of infracted area, and then tracked by BLI and PET/CT during 28 days. They found that this time window can be speculated to be a critical period for cell therapy, providing a platform for cell growth and holding a paracrine role in subsequent cell differentiation to enhance cell-based therapeutic effects. All this could also be explained by better preservation of cardiac function through transplantation of ADSCs with fibrin scaffolds. Furthermore, it is possible to provide potential mechanisms by which injectable biomaterials support improved early graft cell adhesion, reduced inflammation and elimination of oxidative stress, thus paving the way for adequate long-term cell survival and eventual effective improvement. Also, it is particularly important to employ a non-invasive multimodal imaging approach to tracing the in vivo fate of stem cells.

3.4 iPSC and myocardial infarction

iPS cells with the ability to self-renew are expected to be a new potential source of cells for the treatment of many different degenerative diseases, including cardiovascular disease. Transplanted iPS cell-derived cardiac cells have been shown to integrate into the heart undergoing treatment and improve cardiac function. However, the exact mechanism that mediates this effect is currently unknown [35].

Masashi Kawamura et al. [36] have shown that a large number of highly pure hiPS-CMs and those hiPS-CM sheets could improve cardiac function after ischemic cardiomyopathy, primarily because of paracrine cytokine effects. Transthoracic echocardiography was performed during 8 weeks after myocardial infarction. At the last week, cardiac multi-slice CT was executed and then histology, immunohistolabeling and fluorescence were practiced. The main findings of this study are as follows: hiPS-CM slices survived in damaged myocardium in the short term and improved cardiac function in a porcine ischemic cardiomyopathy model; transplantation of hiPS slices attenuated left ventricular remodeling and increased neovascularization; hiPS-derived cardiomyocytes were still detectable 8 weeks after transplantation, but the number of long-term surviving hiPS-CMs was very low. Teratoma formation was not observed in animals that received hiPS-CM slices. In addition, ischemia-related dormancy in the infarct-edge myocardium may be restored by increased blood supply due to angiogenesis, all of which are associated with paracrine effects.

4. Conclusions and future directions

Stem cell therapy has shown exciting promise in patients with acute myocardial infarction or chronic ischemia. Every kind of stem cell has own its distinctive advantages and disadvantages, they would be choose according to need of clinical and preclinical application. However, before a wide clinical translation becomes reality must undergo extensive scrutiny. There are several crucial problems that must be pointed out that safety and ethical issues, the best route for cell delivery, type and quantity of stem cells and the time of inject. Most importantly, to date, the effectiveness of stem cell therapy in terms of both cell survival and proper engraftment in animal models is far from ideal in vivo, multimodality molecular imaging which be used for tracking stem cells long-term fates can offer some information of survival, engraftment and differentiation. Despite of having benefits of multimodality imaging, many process after delivery has been unknown because of the limitation of technology. Advanced imaging technology will likely be provided by future researches and

offer mechanistic insight into regulation of the processes of stem cell therapy. Finally, we absolutely believe that the novel therapy must head for clinical application after extensive appraisal and scrutiny. Imaging can guide each step of stem cell therapy from choosing the appropriate delivery method to evaluating the long-term safety of transplantation. With parallel developments in conventional and molecular imaging technology, stem cell therapy will be applied to clinic gradually.

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Conflict of Interest statement

The authors declare that they have no conflict of interest.

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